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Modelling the kinetics of *Listeria monocytogenes* in refrigerated fresh beef under different packaging atmospheres

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ABSTRACT

The objective of this study was to model the fate of *Listeria monocytogenes* inoculated in beef at two concentrations (2.5 and 4.0 log CFU/g), packaged under air, vacuum and three modified atmospheres MAP: 70%O_2/20%CO_2/10%N_2, 50%O_2/40%CO_2/10%N_2 and 30%O_2/60%CO_2/10%N_2, and refrigerated at a normal temperature (4 °C) and at a mild abusive temperature (9 °C). The experimental design produced a total of 20 environmental conditions. An omnibus model based on the Weibull equation proved statistically that *L. monocytogenes* survives better in vacuum (VP) than in aerobic conditions, although without significant difference in its ability to survive in the temperature range between 4 °C and 9 °C. Furthermore, regardless of the refrigeration temperature, the presence of CO_2 in the package atmosphere exerted a bactericidal effect on *L. monocytogenes* cells, being approximately 1.5 log of reduction when storage time reached 10 days. Since the pathogen can survive in VP/MAP beef, there is a need of maintaining its numbers below 100 CFU/g before packaging by placing efforts on the implementation of control measures during processing.

1. Introduction

Listeria monocytogenes is a Gram-positive anaerobic facultative food-borne pathogen capable to survive in environments with pH between 4.0 and 9.6 (optimum 6.0–8.0), and at water activity (a_w) levels as low as 0.90 (Farber & Peterkin, 1991). Moreover, this pathogen can survive at temperatures below freezing, and can grow from 1 °C to 45 °C. As a consequence, because of this bacterium's ability to proliferate under chilled temperatures, some types of food products have recurrently exhibited more susceptibility to *L. monocytogenes* contamination. In particular, refrigerated meats, ready-to-eat meat (RTE) foods, milk and cheeses, smoked fish and seafood, have been implicated in isolated cases of listeriosis (EFSA, 2015; Martins & Germano, 2011). As *L. monocytogenes* is a microorganism of ubiquitous nature, meat products may become contaminated with this pathogen through raw materials, processing environment and at retail markets (Sofos & Geornaras, 2010).

In 2013, EU reported 1763 confirmed human cases of listeriosis, which represented an 8.6% increase compared to 2012. It was also concluded that there was a statistically significant increasing trend of listeriosis in the EU over the period 2009–2013 (EFSA, 2015). A recent meta-analysis on the incidence of pathogens in Portuguese meats revealed that incidence average of *L. monocytogenes* in beef meat is 17.6%, and in meat products is 8.8% (Xavier, Gonzales-Barron, Paula, Estevinho, & Cadavez, 2014). Although the concentrations are unknown, and may as well be low, these high prevalence estimates may represent a considerable risk due to the common practice among consumers of eating rare meat. Other malpractices resulting in higher *L. monocytogenes* levels, such as cross contamination and storage at abusive temperatures, may also exacerbate the risk for consumers, especially for immunocompromised people (Swaminathan & Gerner-smidt, 2007). According to Santos, Correia, Cunha, Saraiva, and Novais (2005), the limit of 2.0 log CFU/g of RTE was considered unacceptable or potentially dangerous for public health. This limit must not be exceeded at the end of the shelf-life (Commission European Communities, 2008).

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Among the parameters affecting the growth of microorganisms in packaged raw meat, it is assumed that the final pH and the gaseous composition of the packaging have an important role (Zakrys, Hogan, O'Sullivan, Allen, & Kerry, 2008). In red meat packaging, the most common methods are vacuum packaging (VP) and modified atmosphere packaging (MAP). It is well known that the composition of modified atmosphere systems can be an effective way to restrict the growth of spoilage aerobic organisms (McMillin, 2008; Tsigarida, Skandamis, & Nychas, 2000); but their effectiveness strongly depends on storage temperature and film permeability (McMillin, 2008). Nevertheless, the extended shelf-life of refrigerated meats under VP and MAP conditions has raised concerns about the survival dynamics of *L. monocytogenes*. At present, in the literature, there are a series of contradictions related to the effect of VP and MAP on the growth/survival of this pathogen in meats and meat products (Arvanitoyannis & Kotsanopoulos, 2012, Chap. 3; Arvanitoyannis & Stratakos, 2012, Chap. 5; García-de-Fernando, Nychas, Peck, & Ordóñez, 1995; Lyver, Smith, Tarte, Farber, & Nattress, 1998; Uppal et al., 2012). In addition, in the references above, no efforts have been directed to developing a predictive microbiology model that can estimate the dynamics of *L. monocytogenes* in refrigerated packaged meat. Within this context, the objective of this work was to assess, by means of predictive microbiology modelling, the effect of packaging (air, vacuum and three gas compositions of MAP) on the dynamics of *L. monocytogenes* inoculated at low and high level in beef meat stored at a normal refrigeration temperature (4 °C) and a mild abusive temperature (9 °C).

2. Material and methods

2.1. Preparation of meat samples

Samples of *Longissimus dorsi* (LD) muscle were obtained from eight Portuguese bulls aged 9–11 months old, having carcass weights between 90 and 150 kg. LD was excised from the carcasses, between the 6th thoracic and the 2nd lumbar vertebra 24 h *post mortem*. The pH was measured directly in the muscle using a combined glass electrode with a pH-metre (Crison Instruments, Spain) (ISO 2917, 1999). Only muscles whose pH were below or equal to 5.8 were used. The activity of water (a_w) was measured with a rotronic-Hygroskop DT at 25 °C according to ISO 21807 (2004). The a_w of muscles were 0.97 ± 0.01. Muscles were cut into pieces of approximately 200 g and two samples of each piece were immediately (24 h *post mortem*) investigated for the presence of *L. monocytogenes* according to ISO 11290-1 (1996). If at least one positive-sample from each muscle were detected in one meat cut, all piece cuts of the whole muscle were totally excluded from the inoculation experiments. Meat cuts were then packed in vacuum, and kept at −80 °C during 15 days before the experiment. For the experiments, meat was left to defrost overnight at 2 °C. Meat samples were then prepared by removing a layer of ~1 cm from the meat surface, and aseptically cut in small meat pieces (0.5 cm thick, surface 2 × 2.5 cm) of a weight of ~5 g.

2.2. Preparation of inoculum and inoculation procedure

L. monocytogenes (ATCC 7973) stock culture was cultured on tryptone soy agar (TSA, England) slants at 4 °C, which were replaced every 30 days. During the course of the study, this bacteria was sub-cultured by transferring a single colony from the slants into 10 ml Brain Heart Infusion (BHI) broth (Oxoid CM225, England) for 24 h at 37 °C, followed by a second activation step in BHI (37 °C, 18 h) to achieve a viable cell population of 9 log CFU/ml.

The culture was then transferred to a sterile centrifuge bottle and centrifuged at 10,000 × g for 10 min at 4 °C. The supernatant was decanted and the sediment suspended in 0.1% peptone water (Merck, Germany). The washing step was repeated twice. The suspension of washed cells was diluted in a sterile 0.1% peptone solution to obtain an optical density of 0.5 (600 nm, 10 mm). Serial (10-fold) dilutions were performed to yield approximately 2.5 or 4 log CFU/g.

On the day of the experiment, each piece of beef was placed into an individual package and inoculated with 20 μl bacterial suspension of *L. monocytogenes* in the sample's centre. The entire procedure was repeated to obtain a low inoculation level (2.5 log CFU/g) and a high inoculation level (4.0 log CFU/g). Each bag of beef was massaged manually and then bags were heat-sealed. Duplicate beef samples (5 g) inoculated with 20 μl of 0.1% (w/v) sterile peptone water served as negative controls and the bags were heat-sealed.

To verify the number of viable *L. monocytogenes* in the suspension, each inoculum was enumerated by making appropriate dilutions in peptone water (0.1%, w/v; PW) in duplicate, and plating onto TSA plates to obtain the initial population densities. Plates were incubated at 37 °C for 24 h before enumeration.

2.3. Packaging

Inoculated samples were packed in 5 different packaging types, namely: air (A), vacuum (V) and modified atmosphere packaging $70\%O_2/20\%CO_2/10\%N_2$ ($MAP_{70/20}$), $50\%O_2/40\%CO_2/10\%N_2$ ($MAP_{50/40}$) and $30\%O_2/60\%CO_2/10\%N_2$ ($MAP_{30/60}$). For air-packaged samples, meat cuts were accommodated in a tray and overwrapped with polyethylene film, while for vacuum-packaged samples, meat cuts were individually packaged in COMBITHERM bags (WIPAK Walsrode, HAFRI) of 0.09 mm thickness, oxygen transmission rate (OTR) of 63 $cm^3m^{-2}day^{-1}$ at 23 °C/0% RH, and water vapour transmission (WVT) of 1 g $m^{-2}day^{-1}$ at 23 °C/85%RH. For modified-atmosphere packaged samples, beet cuts were individually placed in COMBITHERM XX bags (WIPAK Walsrode, HAFRI) of 0.115 mm thickness, a very low O_2-permeable film (OTR of 1 $cm^3m^{-2}day^{-1}$ at 23 °C/0% RH) with a WVT of 1 g $m^{-2}day^{-1}$ at 23 °C/85%RH. The meat samples were packaged either in modified atmosphere or vacuum using a SAMMIC V-420 SGA machine, with a final gas-to-meat ratio of ~3:1. Individual samples were then stored at 4 ± 0.5 °C and 9 ± 0.5 °C, and examined for *L. monocytogenes* counts (ISO 11290-2, 1998) at days 1 (2 h after packaging), 3, 7, 10, 14, 21 and 28, depending on the packaging system.

2.4. Microbiological analysis

At each time point, two samples were prepared for microbiological analysis. The meat cuts were homogenised with 20-ml sterile buffered peptone water (BPW) in a stomacher (IUL, Barcelona, Spain) during 90 ss at room temperature. Serial decimal dilutions were prepared in BPW, and duplicate 0.1 ml samples were then plated onto Compass *L. monocytogenes* agar (Biokar BM06508). After incubation at 37 °C for 48 h, typical colonies were counted and results were expressed as log CFU/g.

Serial decimal dilutions of control samples were also prepared in BPW, and duplicate 1 ml samples were plated onto selective CFC (Cetrimide, Fucidin, Cephaloridine) Agar (OXOID CM0559) with CFC selective supplement (OXOID SR0103) for *Pseudomonas* spp. (25 °C for 48 h, NF V04–503, 1988) and 1 ml samples were plated on double layer on MRS agar (OXOID CM361) for LAB counts (37 °C, 24 h) (NF V04–504, 1998).

The enumeration of *Pseudomonas* spp. (NF V04–503, 1988) was completed by biochemical test in KLIGLER (OXOID CM0033) and oxidase test. Results were expressed as log CFU/g. In case the microorganism counts were below to the detection limit, the result